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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of  
Komei WASHINO et al.

Serial No.: 09/744,550

Group Art Unit: 1617

Filed: January 26, 2001

Examiner: Lauren Q. WELLS

For: DRUGS FOR MEDICAL USE ENABLING NUCLEAR MAGNETIC  
RESONANCE DIAGNOSIS BY SCALAR COUPLING

#14  
HKO  
4-23-03

D E C L A R A T I O N

Commissioner for Patents  
Washington, D. C. 20231

Sir:

Akira NAKATANI, a Japanese citizen, c/o  
Soyakukenyusho, NIHON MEDI-PHYSICS CO., LTD. of 3-1,  
Kitasode, Sodegaura-shi, Chiba, Japan, hereby solemnly  
and sincerely declares:

That I am one of the co-inventors of the  
above-identified application;

That I have read and understand the Official  
Action and the prior art references cited in the  
Official Action dated October 17, 2002;

That in order to demonstrate that according to  
the drug composition of the present invention, the

biodistribution of the O-17 containing water as a solvent in vivo varies depending on the constituents of the drug, and the biodistribution can be detected by nuclear magnetic resonance, I conducted the following additional experiments:

Additional Example 1

- (1) Preparation of 5 atm% O-17 water-containing D-mannitol

11.428 g of D-mannitol (extra pure agent,  $C_6H_{14}O_6$ , Mw. 182.17, Lot. MOH5866, mfd. by NacalaiTesque, Inc.) was dissolved in injection solvent of water so as to obtain 50 mL of the solution. 10.5 mL of the solution was taken and 1.5 mL of 40 atm% O-17 water was added to the solution, so that 12 mL of 5 atm% O-17 water-containing D-mannitol aqueous solution was prepared.

- (2) Administration of the 5 atm% O-17 water-containing D-mannitol to rats

Under anesthesia using isoflurane, 4 mL of the 5 atm% O-17 water-containing D-mannitol thus prepared was administered by injection to three rats (rat Nos. 1 to 3) from each caudal vein, at the rate of 0.5 mL/sec (4 mL/8 sec).

### (3) Preparation for specimens of organs

#### i) Organ extraction

Each of three rats was anesthetized with Ravonal before the extraction of organ. Thirty minutes after the administration, rats made bleeding to death, then the organs such as brain, liver (folio), kidney (one-sided), muscle (femoral region) and blood were excised from rats. These organs excised put into the well-closed containers, so as to prevent it from drying out.

#### ii) Preparation for organ homogenized specimens

Each of the organs was dissolved to obtain the sustained solution for NMR measurement as follows:

##### a) Material for use

Kit for protein decomposition: Getpure DNA  
Kit-cell Tissue (code: GK03, mfd. by Dojinndo Molecular technologies Inc.)

Centrifugal tube: 2 mL tube

Incubator: BW 101 and BF 200 (mfd. by YAMATO)

Scale: METTLER AT 400

##### b) Dissolving method

The weight of the empty centrifugal tube was measured, and then each of the excised organs was chopped into small specimens and 60 to 70 mg of the

small specimens was put into each of the centrifugal tubes.

The total weight of the tube with specimens was measured. Further, 800  $\mu$ L of lysis buffer and 20  $\mu$ L of Protein K solution were added thereinto and the total weight of the tube was measured.

The centrifugal tube was sealed with tape. The content of the tube was well mixed by Voltex and incubated at 55°C. The incubation was continued for 72 hours till the dissolution was confirmed by visual observation.

#### (4) NMR measurement

The homogenized solution of the excited organs was put into a 5mm  $\phi$ -NMR tube, and measurement by NMR equipment (JNM-ECP500, mfd. by JEOL) was conducted under the following conditions: observation frequency = 67.8MHz (O-17 nuclear resonance frequency), accumulator number = 5000, sweep width = 40.8kHz, sampling point = 2048 and pulse width = 45°.

The result is shown in Table 1 below.

Additional Example 2

- (1) Preparation of 5 atm% O-17 water-containing "KN replenisher 4A"

0.3531 g of sodium lactate (abt. 72.5%,  $\text{CH}_3\text{CH}(\text{OH})\text{COONa}$ , Mw. 112.06, Lot. MLN9820, mfd. by Wako Pure Chemical Industries, Ltd.); 11.428 g of D-(+)-glucose anhydrous (guaranteed reagent,  $\text{C}_6\text{H}_{12}\text{O}_6$ , Mw. 180.16, Lot. M4T2139, mfd. by Han-i Chemical Industries, Ltd.); and 0.2674 g of sodium chloride (guaranteed reagent,  $\text{NaCl}$ , Mw. 58.44, Lot. 6225) were dissolved in injection solvent of water so as to obtain 200 mL of the solution. 10.5 mL of the solution was taken and 1.5 mL of 40 atm% O-17 water was added to the solution, so that 12 mL of 5 atm% O-17 water-containing "KN replenisher 4A" aqueous solution was prepared.

- (2) Administration of the 5 atm% O-17 water-containing "KN replenisher 4A" to rat

Under anesthesia using isoflurane, 4 mL of the 5 atm% O-17 water-containing "KN replenisher 4A" thus prepared was administered by injection to three rats (rat Nos. 4 to 6) from each caudal vein, at the rate of 0.1 mL/sec (4 mL/40 sec).

Preparation for specimens of organs and NMR measurement were identical with those of additional Example 1 mentioned above.

The result is shown in Table 2 below.

Further, the O-17 concentration in each organ obtained from the additional Examples 1 and 2 are summarized in Fig. 1 shown below.

**Table 1**

<5 atm% O-17 water-containing D-mannitol>

		brain	liver	kidney	muscle	blood
rat No. 1	Integral value for natural abundance of O-17: A	18.74	18.74	18.74	18.74	18.43
	Integral value for homogenized solution: B	22.52	21.20	20.92	22.61	22.45
	Rate of the integral values (B/A)	1.202	1.131	1.116	1.207	1.218
	O-17 concentration in homogenized solution (atm%)	0.046	0.043	0.042	0.046	0.046
	Dilution rate at the preparation of specimens	0.080	0.084	0.074	0.082	0.078
	<b>O-17 concentration in organ (atm%/g)</b>	<b>0.572</b>	<b>0.509</b>	<b>0.573</b>	<b>0.559</b>	<b>0.590</b>
rat No. 2	Integral value for natural abundance of O-17: A	18.74	18.74	18.74	18.74	18.43
	Integral value for homogenized solution: B	21.77	21.11	22.18	21.74	22.53
	Rate of the integral values (B/A)	1.162	1.126	1.184	1.160	1.222
	O-17 concentration in homogenized solution (atm%)	0.044	0.043	0.045	0.044	0.046
	Dilution rate at the preparation of specimens	0.083	0.082	0.081	0.086	0.091
	<b>O-17 concentration in organ (atm%/g)</b>	<b>0.534</b>	<b>0.525</b>	<b>0.558</b>	<b>0.511</b>	<b>0.508</b>
rat No. 3	Integral value for natural abundance of O-17: A	18.74	18.74	18.74	18.74	18.43
	Integral value for homogenized solution: B	22.31	22.03	22.91	21.46	22.60
	Rate of the integral values (B/A)	1.191	1.176	1.223	1.145	1.226
	O-17 concentration in homogenized solution (atm%)	0.045	0.045	0.046	0.044	0.047
	Dilution rate at the preparation of specimens	0.078	0.078	0.076	0.076	0.085
	<b>O-17 concentration in organ (atm%/g)</b>	<b>0.580</b>	<b>0.576</b>	<b>0.611</b>	<b>0.575</b>	<b>0.547</b>
O-17 average concentration in organ (atm%/g)		0.562	0.537	0.581	0.548	0.548

(Natural abundance of O-17 = 0.038 atm%)

**Table 2**

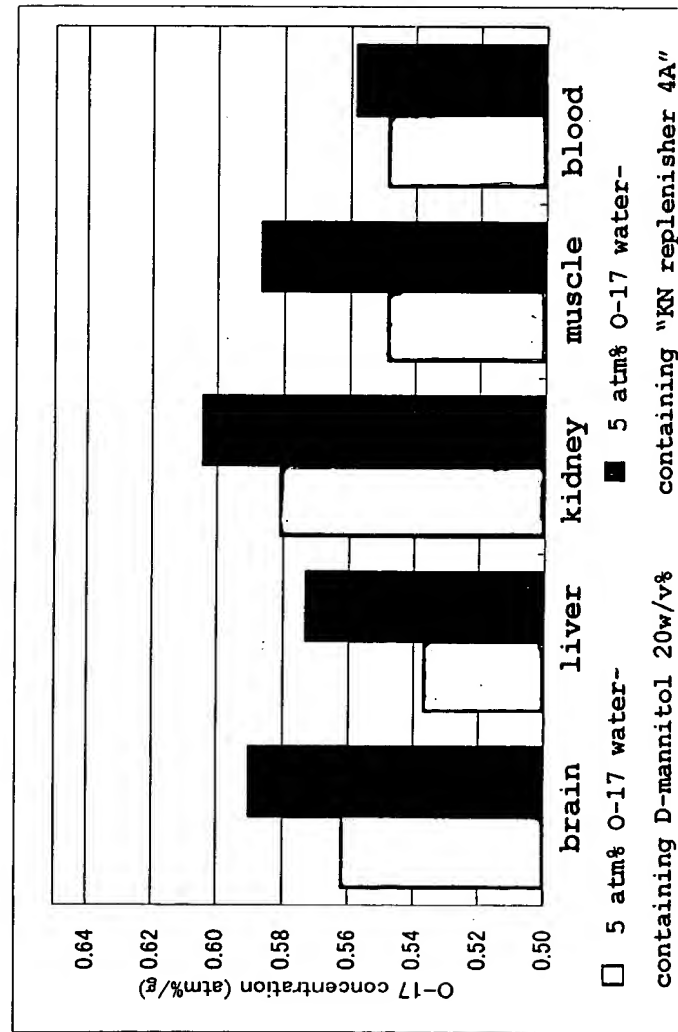
<5 atm% O-17 water-containing "KN replenisher 4A">

rat No.		brain	liver	kidney	muscle	Blood
4	Integral value for natural abundance of O-17: A	18.74	18.74	18.74	18.74	18.43
	Integral value for homogenized solution: B	22.41	20.83	20.95	22.46	22.27
	Rate of the integral values (B/A)	1.196	1.112	1.118	1.199	1.208
	O-17 concentration in homogenized solution (atm%)	0.045	0.042	0.042	0.046	0.046
5	Dilution rate at the preparation of specimens	0.073	0.072	0.075	0.075	0.083
	<b>O-17 concentration in organ (atm%/g)</b>	<b>0.622</b>	<b>0.590</b>	<b>0.569</b>	<b>0.605</b>	<b>0.551</b>
	Integral value for natural abundance of O-17: A	18.74	18.74	18.74	18.74	18.43
	Integral value for homogenized solution: B	22.14	21.43	21.67	22.42	23.46
6	Rate of the integral values (B/A)	1.181	1.144	1.156	1.196	1.273
	O-17 concentration in homogenized solution (atm%)	0.045	0.043	0.044	0.045	0.048
	Dilution rate at the preparation of specimens	0.074	0.077	0.071	0.074	0.088
	<b>O-17 concentration in organ (atm%/g)</b>	<b>0.603</b>	<b>0.566</b>	<b>0.618</b>	<b>0.611</b>	<b>0.547</b>
	Integral value for natural abundance of O-17: A	18.74	18.74	18.74	18.74	18.43
	Integral value for homogenized solution: B	22.44	21.35	22.62	21.65	22.74
	Rate of the integral values (B/A)	1.197	1.139	1.207	1.155	1.234
	O-17 concentration in homogenized solution (atm%)	0.046	0.043	0.046	0.044	0.047
	Dilution rate at the preparation of specimens	0.083	0.077	0.073	0.081	0.081
	<b>O-17 concentration in organ (atm%/g)</b>	<b>0.545</b>	<b>0.562</b>	<b>0.626</b>	<b>0.545</b>	<b>0.576</b>
	O-17 average concentration in organ (atm%/g)	0.590	0.573	0.604	0.587	0.558

(Natural abundance of O-17 = 0.038 atm%)

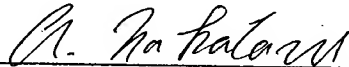


Fig. 1



The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 26th day of March, 2003.



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Akira NAKATANI